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Nervous Connections of the Mammalian Spleen, including an account of certain Visceromotor and other Abdominal Reflexes.

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NERVOUS CONNECTIONS OF THE MAMMALIAN SPLEEN, INCLUDING AN ACCOUNT OF CERTAIN VISCEROMOTOR AND OTHER ABDOMINAL REFLEXES.

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(With eight figures.)

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The results here recorded form part of the data obtained in a more general investigation of splenic function—see Tait and Cashin (1925), E. S. Mills (1926), and Tait and Mills (1927).

Literature.

Efferent Nerves causing Contraction.—Schiff (1867) was the first to show that stimulation of the splanchnic nerves and of the cœliac ganglion causes contraction of the spleen. Bulgak (1877) found that stimulation of anterior root fibres over a considerable extent of the thoracic cord causes contraction of the spleen by way of the great splanchnic nerve. Roy (1881) introduced the plethysmograph for study of splenic contraction. He observed that the organ is normally subject to regular alternation of volume, in virtue of which it acts as a pump. Schäfer and Moore (1896), also using a plethysmographic technique, were able to confirm and to extend many of Bulgak's results. They found that the muscular tissue of the spleen derives a spinal innervation from a very large number of nerve roots, viz. from the 3rd to the 14th post-cervical segment in the dog and in the cat. The main outflow occurs on the left side, and chiefly from the 5th to the 9th post-cervical segment inclusive. The nerve-fibres of supply pertain to the sympathetic autonomic system, which these workers believed to synapse partly in the ganglia of the sympathetic chain, partly in the cœliac ganglion.

Brodie and Halliburton (1902), in an investigation on the fatigability of post-ganglionic non-medullated fibres, made use of the splenic nerve.

Tait and Cashin (1925) pointed out that the spleen is divided into a number of segmental zones, which correspond to the ultimate branches of the splenic nerve entering the organ along with the arteries, and

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proved that these zones are at the same time nervous and arterial units. Kyber (1870) had previously indicated the existence of arterial, and Brodie and Halliburton (1902) the existence of nervous zones. Tait and Cashin likewise found that dilatation of the spleen occurs on stimulation of the splenic vein.

By plethysmographic study of the spleen in dogs which had recovered from the operative interference of the attachment of the plethysmograph, Hargis and Mann (1925) confirmed the previous work of Roy and of Schäfer and Moore in reference to the rate of rhythm of the spleen, and found that the organ shows the greatest amount of contractile and expansile activity during digestion. They were able to prove not only that a denervated portion of the spleen exhibits this rhythmic action to a less degree, but also that various forms of sudden sensory stimulation, especially if not expected by the animal, cause a prompt contraction of the spleen, which depends upon the integrity of its efferent nerves. A somewhat similar study of the spontaneous changes in volume of the spleen was made by Barcroft, Harris, Orahovats, and Weiss (1925), who adopted the device of marking the external limits of the organ with metal clips, which were then photographed on X-ray plates. The previous results of Barcroft and other co-workers (1925) had demonstrated the high importance of the spleen as a reservoir of blood available during emergencies.

Roy (1881) claimed that the vagus as well as the splenic nerve acts as a constrictor to the spleen. Schäfer and Moore having found reason to doubt this finding, Magnus and Schäfer (1901) subsequently proved that the vagus carries neither motor nor inhibitory fibres to the spleen.

Dilator Effects.—In one solitary instance Schäfer and Moore, by the use of slow and weak stimulation, were able to record a dilator effect on stimulating the splenic nerve. A similar effect occurred on ordinary stimulation of the same nerve some hours after section. These seem to be the only uncontroverted records that indicate the possible existence of dilator fibres in the splenic nerve.

The Double Musculature of the Spleen.—There are within the spleen two distinct sets of muscle fibres, (1) that of the trabeculæ, and (2) that of the arteries; and investigators have naturally speculated whether they may come into separate action, or whether they act concomitantly. There is some evidence for the existence of a functional separation between them.

Schäfer and Moore supposed that the splenic contraction caused by suprarenal extract might be due to direct action on the blood-vessels or complicated by an action upon the splenic muscular tissue. De Boer and Carroll (1924*a*) adduced certain facts in support of the belief that the decrease in volume of the spleen which follows CO poisoning is due to a contraction of the splenic muscle, and that the arteries of

the spleen are not constricted. The same authors (1924*b*) were able to establish that whereas adrenin causes a contraction of both the blood-vessels and the capsule of the spleen, pituitrin acts only upon the plain muscle of the blood-vessels and not upon the other muscular tissue.

Tournade and Chabrol (1924) also made investigations which seemed to bear on this question. They found that while a small dose of adrenin causes a rise of general blood-pressure and a contraction of the spleen, a still smaller dose causes contraction of the spleen without contraction of the general arterioles. Their inference was that the intrinsic muscle of the spleen is more sensitive than the arterioles to the action of adrenin. In all recent work it is recognised that in the possession of arterioles the spleen resembles other organs, but that it differs from other organs in exhibiting at once a high degree of rhythmic activity, and a musculature which is peculiar to itself; whence it would seem to follow that the special musculature is responsible for the characteristic rhythmical activity.

The Central Nervous Control of the Spleen.—Bulgak, finding that excitation of the spinal cord in the upper cervical region caused contraction of the spleen, whereas stimulation of the cord at lower levels caused less effect, inferred the existence of a reflex centre for splenic contraction in the upper cervical cord. De Boer and Carroll (1924*b*), on the other hand, concluded that the centre or centres which respond to CO poisoning by effecting contraction in the spleen are situated in the spinal cord below the level of the third cervical vertebra. von Tarchanoff (1874), Bulgak (1877), and also Roy (1881) found that stimulation of the medulla oblongata causes contraction of the spleen.

The Splenic Vein in Relation to Dilatation.—On general grounds it may be said that if there are motor fibres to the smooth muscle of the spleen, so there may well be inhibitory fibres too. In the light of Tait and Cashin's discovery of the marked contractility of the splenic vein it is easily possible to suppose that dilatation may depend as much on a primary contraction of the vein as on a simple inhibition of the muscle in the trabeculæ and the arteries.

Constrictor fibres are known to be distributed to certain veins of the body. Thus Pal (1888) found that stimulation of the splanchnic nerve causes constriction of the portal vein. W. H. Thompson (1893) showed that constrictor nerves pass by way of the sciatic nerve to the saphenous vein. Gunn and Chavasse (1913) found that many veins of the body contract under the influence of adrenin. Donegan (1921) established the presence of constrictor nerves to the superficial veins of the fore- and hind-limbs and to the mesenteric veins of different laboratory animals. Hooker (1918) extended these results to the sigmoidal flexure of the dog. Horiuchi (1924) established the presence of dilator fibres to certain veins. The effect noted by Tait and Cashin on the

vein might therefore have been due to a primary stimulation of constrictor nerve-fibres.

Afferent Fibres in the Splenic Nerve.—Whereas the earlier experiments on the sympathetic nervous system seemed to show the presence only of efferent fibres, later work has demonstrated the presence of afferent nerves as well.

Langley (1900) showed that afferent neurones connected with the viscera are situated in the dorsal spinal ganglia. Sherrington (1909) pointed out that stimulation of the central end of a branch of the solar plexus caused by reflex action an immediate contraction of abdominal muscles. Mackenzie (1918) laid stress on the fact that the abdominal muscles tend to become rigid when certain abdominal viscera are affected by disease. This increase of rigidity he termed a visceromotor reflex. By stimulating the central end of nerves proceeding from the cœliac ganglion, and recording the corresponding contractions of the abdominal muscles, Miller and Simpson (1924) studied some of these visceromotor reflexes. They mention that stimulation of the splenic nerve causes contraction of these muscles, especially those of the left side. Thus it is plain that there are afferent fibres in the splenic nerve.

Experimental.

Technical Details.—The animals used for experiment were dogs and cats. The usual anæsthetic was amytal, which was injected intraperitoneally in doses of 0·5 to 0·7 c.c. of a 10 per cent. solution per kilo. To expose the spleen with its immediate nervous connections the abdomen was opened and four or five ribs were resected on the left side. As this latter manœuvre involved opening the chest, artificial respiration was maintained with a Jacquet pump. After exposure of the cœliac ganglion the main nervous trunks proceeding from it towards the spleen were isolated, and labelled according to their anatomical relation to the splenic artery. In the dog three or more of these trunks can be identified; in the cat they are too small for easy separation.

For plethysmographic records air plethysmographs were used: the recording apparatus was of special design, and consisted of a varnished metal trough containing mercury over which floated, somewhat as in a gasometer, a light, air-filled celluloid box of special shape. This box was hinged at one side, and moved after the fashion of a bellows recorder, its excursions being appropriately magnified by a lever. The linear record of this apparatus represented with fair accuracy the actual volume changes that occurred. For simple (qualitative) demonstration of expansion and contraction a Marey tambour was used.

To obtain a permanent record of the change in form of the surface of the organ under different conditions we adopted the plan of pressing against the surface of the spleen a piece of smoked paper. Just as the

ink on a printer's block transfers to paper a picture of the block, so the splenic surface detaches soot from the smoked paper, and leaves an impression not only of its size, but in many cases of its little roughnesses, its elevations and depressions. This method proved of some service in recording not only gross changes in size, but also changes in the character of muscular contraction within the organ.

In all experiments on the exposed spleen it is particularly necessary to avoid traction on the mesentery or other mechanical stimulation of the adjacent viscera, which by reflex action cause a contraction of the organ.

Efferent Nerves causing Contraction.—The general observations of Tait and Cashin were repeatedly confirmed. The nerves that enter with the terminal arteries are so fine that in order to stimulate them it is necessary to place the electrodes on the artery. Dissection under the binocular microscope later showed that the number of nerve branches entering the organ is greater than the number of arteries. Consequently the result of placing the electrodes on the artery is to get a combined effect due to the stimulation of more than one nerve filament.

When one examines the splenic nerve in its relation to the main splenic artery, one finds that the nerve is not single, but consists of trunks which are differently disposed in relation to the blood-vessel. Two conspicuous trunks can usually be distinguished: one (which we shall here call trunk A) on the caudad aspect of the artery; the other (trunk B) on the opposite side of the vessel, *i.e.* on the craniad aspect. These trunks may be single; frequently one or both of them may consist of a pair of parallel bundles which anastomose at intervals. The nerve trunks, along with the artery itself, are enclosed in a loose connective-tissue sheath somewhat like the carotid sheath. They are thus in contact with, but are not incorporated in, the adventitial coat of the artery.

At the point of bifurcation of the main artery each of the two nerve trunks similarly divides and sends a branch to accompany each arterial branch. A point of interest is that the nervous branches again lie on opposite sides of the artery, and in such a way that the branch from trunk A remains caudad, that from trunk B craniad. A similar redistribution of the nerves occurs when new arterial branches arise, so that any given arterial branch is always accompanied by two separate nervous filaments, one derived from trunk A, the other from trunk B (fig. 1). When the terminal arteries enter the hilus of the spleen the branch from trunk A enters with the artery, to which it closely clings; on the other hand, the ultimate branches from trunk B enter along the line of the hilus, but not in such close relation to the arteries. This curious anatomical arrangement, twice verified by dissection, naturally suggests some functional difference between the fibres of trunk A and those of trunk B. One might, for example, suppose that the fibres

of trunk A are vasomotor, while those of trunk B may represent the fibres to the trabeculæ. On the other hand, one set might be efferent, the other afferent.

Still a third nerve-trunk C, lying ventrally between trunks A and B, may sometimes be traced for some distance along the main splenic artery. Because of its minute size it is more hard to follow this trunk to its final destination, and it may possibly part company from the artery and join the splenic vein; at the same time it is equally possible that it accompanies the artery throughout.

The most satisfactory way of investigating the individual function of nerve-trunks A and B is to expose these trunks not on the main

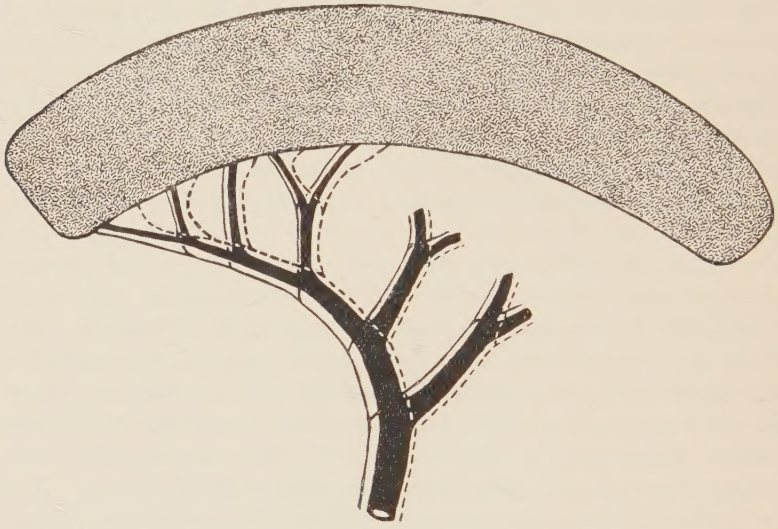

Fig. 1.—Diagram of splenic artery of dog, to show how a branch of nerve-trunk A and another of nerve-trunk B accompany each sub-division of the artery.

splenic artery, but on one of the primary branches beyond the first bifurcation. Under these conditions it is possible to test the action of the nerves on a portion of the spleen, the half which is supplied by the other arterial branch meanwhile remaining unaffected and serving as a control. In actual practice it is more easy to isolate the more medial of these two arterial branches. Thus any effect due to nerve stimulation is observed upon the medial "half" of the spleen, which in turn is the portion of the organ with the longest mesentery, and, therefore, most easy of access and of observation. After contraction of the spleen, due to faradic stimulation of any particular nerve-trunk, it is necessary to allow a rest period of five or ten minutes in each case until the organ dilates again. During this time the spleen is kept covered with cloths wrung out of warm saline.

Experiment.

Medium-sized dog. Amytal anæsthesia. After resection of ribs the spleen was lifted so as to expose the caudad aspect of the mesentery. The more medial of the two primary arterial branches was freed of its coverings, and nerve-trunks A and B were defined. Each was found to consist of two parallel filaments, which were separately labelled A_1, A_2, B_1, B_2. In this particular case trunk C was also defined and labelled.

A print of the spleen was first taken in its relaxed condition (fig. 2). Next, filament B_1 was stimulated. The result was a strong contraction of the medial half of the spleen, the surface of which showed a finely granular appearance (characteristic, according to MALL, 1900, of contraction of the trabeculæ). After a suitable rest period, filament B_2, somewhat smaller in size, was stimulated. A similar strong contraction occurred, this time involving only a segment towards the middle of the organ. When filaments A_1 and A_2 were similarly stimulated, the result was in each case a partial contraction of the whole medial half of the spleen, the surface this time showing a more coarsely lobular appearance. (For an illustration of such an effect see fig. 3.) Finally, when trunk C was stimulated the spleen dilated somewhat.

Altogether eight experiments of this nature were carried out. They varied somewhat in detail and in result. At the beginning of these experiments we were unaware of the peculiar anatomical arrangement whereby each arterial branch receives filaments both from trunk A and from trunk B. Consequently the electrodes were not always applied with that degree of care and discrimination that eventually proved to be necessary. So soon as the characteristic anatomical distribution became known to us, the experiments were repeated with the plethysmograph. A series of four plethysmographic experiments then showed that stimulation of trunk B always causes a greater total contraction than stimulation of trunk A. Taking these results in conjunction with the finding that the final filaments of trunk A accompany the arteries, whereas those of trunk B enter the hilus separately, we have provisionally inferred that trunk A is destined for supply of the arteries and trunk B for supply of the trabeculæ.

Dilator Effects.—It has already been mentioned that stimulation of trunk C causes dilatation. This finding was confirmed on a few occasions. As to the ultimate destination of this nerve, we have no information to offer. Dilatation was never observed as a result of stimulating either trunk A or trunk B.

Innervation of the Splenic Vein.—It was soon discovered that special nervous filaments of extreme tenuity are distributed to the splenic vein. Because of the difficulty of dissecting these filaments in preserved preparations their origin and complete course could not be

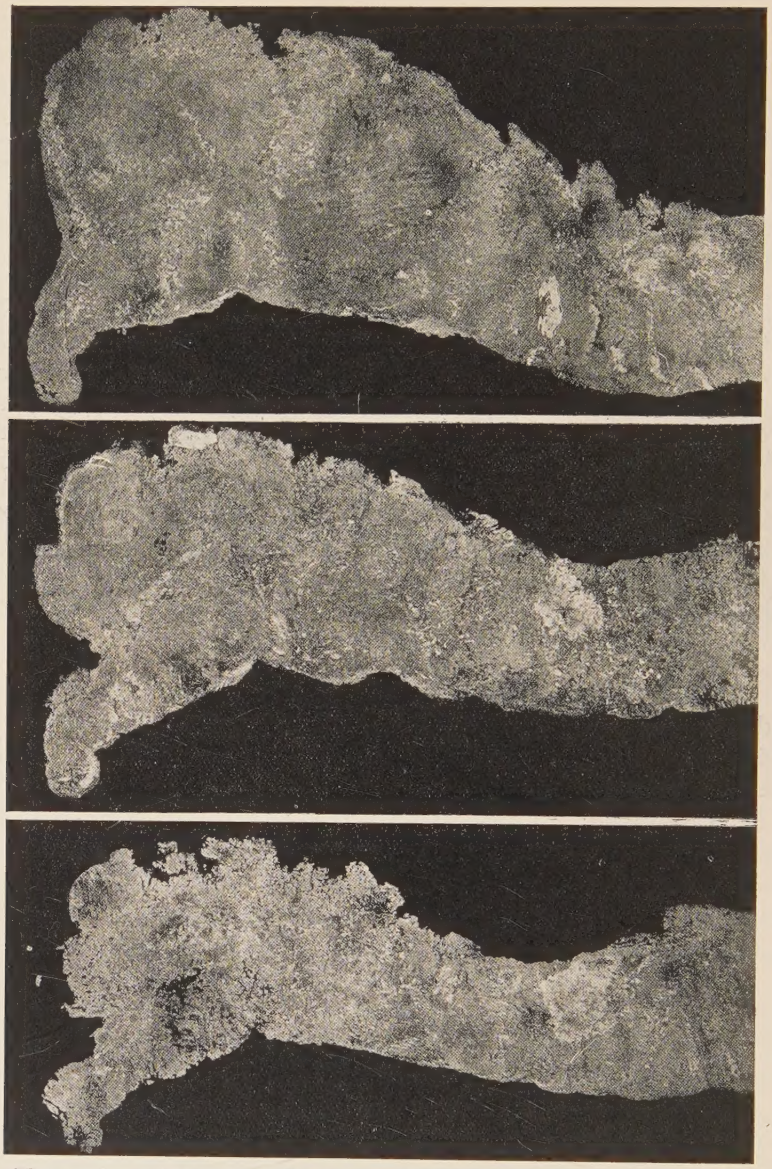

FIG. 2.—Three prints of the spleen of a dog, to show the effect of stimulation of individual nerve-trunks accompanying the medial division of the splenic artery. The prints were taken by pressing smoked paper on the surface of the organ.

The upper print, showing the major part of the medial "half" of the spleen, exhibits the organ in a relaxed condition. The middle print, showing the whole of the medial "half," exhibits the effect of stimulation of nerve-trunk A_1. The lower print, showing the medial "half" and a portion of the lateral "half," gives the result of stimulation of nerve-trunk B_1.

N.B.—(1) After stimulation of trunk B_1 the spleen is more shrunken than after stimulation of trunk A_1; (2) after stimulation of B_1 the lateral, unstimulated "half" of the spleen remains unaffected, as it also was after stimulation of A_1, though, owing to trimming of the tracings for reproduction, none of the lateral "half" appears in the middle print.

determined as in the case of the nerves accompanying the artery. Their presence on isolated lengths of the living vein could, however, be proved by experiment. Such filaments, or such nerves, were found to lie on the side of the main splenic vein, and also to enter the primary and secondary branches of the vein. Thus their distribution is extensive and not restricted. Furthermore, by stimulation of these filaments it is possible to cause contraction, at one time of the main vein, at another time of a primary branch, and still again of secondary branches.

To reach these nerves as they lie beside the main vein it is necessary to perform a left-sided thoracoplasty, to throw the spleen caudalwards,

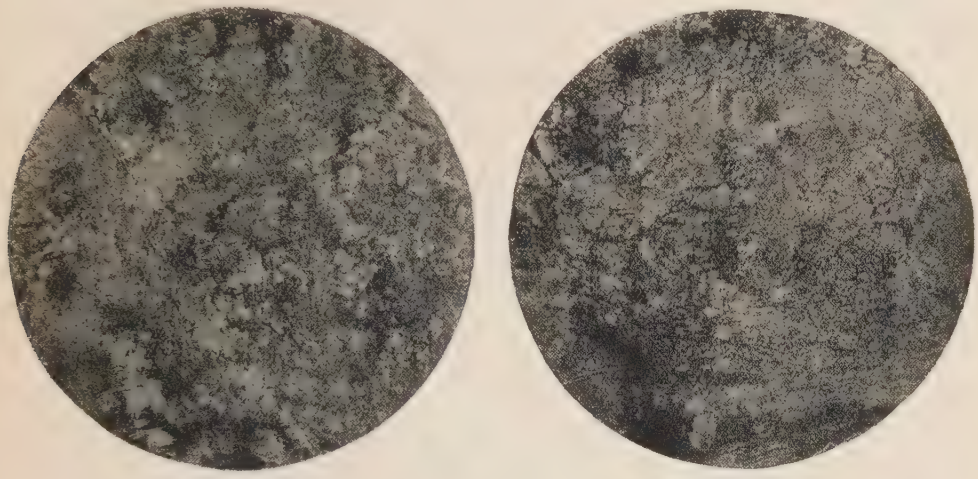

Fig. 3.—Two natural-size prints of one and the same portion of splenic surface, to illustrate the difference in effect produced by stimulation of nerve-trunk A (arteriomotor ?) and of nerve-trunk B (trabeculomotor ?). The prints were taken by pressing smoked paper on the surface of the organ.

The illustration on the left, corresponding to stimulation of trunk A, exhibits a number of coarse blotches, the surface being pebbly. That on the right, corresponding to stimulation of trunk B, shows very numerous fine granulations, the surface being more like that of sand.

to incise the gastro-splenic omentum, and, by carefully removing the peritoneal covering of the splenic mesentery, to expose the vein from its first bifurcation to its point of entry into the portal vein. Using this route one avoids the main splenic artery with its nerves. To expose the nerves as they lie in relation to the branches of the vein it suffices to throw the spleen towards the diaphragm, and to proceed as in exposing the nerves to the arteries. Great care is, however, necessary in removing the peritoneum which covers the vessels. A nervous filament may then be found which, coursing along the caudad aspect of a primary venous branch, sends a twig to it, and dividing at a secondary venous branching, terminates by forming in each a fine arborisation near the origin of the secondary venous branches.

Experiment revealed an interesting relation of these nervous filaments to the vein. When a trunk, coursing along a vein, is lifted up and stimulated, the vessel contracts, not at the level of application of the electrodes, but at a point somewhat distal to this. The region involved in contraction may vary from 3 mm. to 1 cm. in length, and a nervous twig is always found to enter here and form a fine arborisation. When a main venomotor trunk is stimulated, contraction may thus occur at isolated parts, including the basal portions of secondary branches. Intervening portions of the vein are less contractile. If the vein is stripped bare of its nerves it contracts at first, but soon undergoes marked dilatation, and is no longer responsive to stimulation by direct application of the electrodes. From this we conclude that the contractions observed by Tait and Cashin resulted from indirect rather than from direct stimulation of the muscle. In this connection it is also worthy of note that, if the venomotor trunk is simply cut across at one particular level, the part of the vein beyond this dilates, and may exhibit a marked contrast in thickness with the more proximal (still innervated) part of the same vein.

In carrying out these experiments certain precautions proved to be necessary. Apart from keeping the operating table warm, and protecting the exposed parts by means of cloths wrung out of warm saline, it is essential to handle the vein with care. Removal of its peritoneal covering invariably causes a temporary constriction of the vessel, and time must be allowed for this to pass off. The isolation of the nerve and the application of the electrodes also require care. The changes in calibre of the vein are always sufficiently great to be detected with the eye; at the same time they were regularly measured with screw calipers. Simultaneous records of the caliper measurements of the vein and of the surface of the spleen were made on smoked paper immediately before and after each venomotor stimulation. A recovery interval of five to ten minutes was allowed between stimulations. In each experiment a sufficient number of observations was made to preclude the possibility of error due to other undetermined (*e.g.* central) conditions. To record by means of the plethysmograph the changes in splenic volume due to simple venomotor stimulation is not easy, because the vein is apt to be compressed when the plethysmograph is sealed.

Experiment.

The venous branch to the medial half of the spleen was exposed from the primary bifurcation to a point 1 cm. below the secondary bifurcation. The operation of removing the peritoneum from the vein caused the vessel to contract from 7 mm. to 4·5 mm. in diameter. After a rest of five minutes the vein had again dilated to 7 mm. Electrodes being now applied to the vein, it was subjected to faradic stimulation for 10 seconds. A segment of vein, extend-

ing from the point of application of the electrodes to a distance of 1 cm. in the distal direction, thereupon contracted to 4 mm. On the proximal side of the electrodes the vein was not affected.

A venomotor filament was now identified coursing along the caudad border of the vein and giving origin to three branches, viz. one to the middle of the primary venous branch and one to the proximal part of each of its two secondary divisions. Stimulation of the nervous branch to the middle part of the vein caused a band of contraction 1 cm. long, extending distally from the point of entry of the nerve into the vein. The change of diameter was from 6·5 to 5·0 mm. At the same time the medial half of the spleen was found to dilate, a tranverse measurement between two selected points increasing from 62 to 66 mm. Five minutes later a similarly induced contraction of the vein, in which its diameter changed from 6·3 to 4·5 mm., caused a linear increase of the same portion of the spleen from 62 to 69 mm. When the nerve was stimulated at 2 cm. proximal to the secondary bifurcation, yet beyond the point of departure of the branch just mentioned, a small band of contraction about 3 mm. long was observed immediately beyond the bifurcation on each of the two secondary venous branches. The primary venous branch remained unaffected. Before stimulation the two secondary venous branches measured 4 mm. each in diameter. One contracted to 3 mm., the other to 2·5 mm. Meantime the aforesaid linear dimension of the spleen changed from 53 to 65 mm.

The essential facts brought out in the above experiment were repeatedly verified. Some variation in the degree of contraction and in the length of vein involved in that contraction was observed in different individuals, but in every case the kind of effect was the same, and dilatation of the spleen always accompanied contraction of the vein. One point of experimental detail perhaps deserves mention at this juncture, viz. that splenomotor filaments derived from trunk B frequently cross the veins near their secondary bifurcations. Stimulation of these nerves produces not dilatation but a segmental contraction of the spleen. An example of an experiment on the main splenic vein remains to be given.

Experiment.

The vein having been reached by the special route described a few pages back, a venomotor nerve coming off one of the parallel divisions of trunk B, 1·5 cm. distal to the union of the splenic vein with the portal, was found to course along the vein and distribute two branches to the main vein. Stimulation of either of these nerve branches produced a local contraction of the main vein, accompanied by engorgement of the spleen. (These observations were repeated eighteen successive times.) Stimulation of the division of trunk B proximal to the point of departure of the

venomotor nerve produced simultaneously contraction of the vein and marked contraction of the middle portion of the spleen, the rest of which, however, became engorged. On subsequent dissection the division of trunk B, which gave rise to the venomotor nerve, was separately traced to the dorsal aspect of the cœliac ganglion.

Still another point of interest is the fact that when plethysmographic records were taken during the time of venomotor stimulation an increase of the volume of the spleen was recorded, whose duration ($1\frac{1}{2}$ to 3 min.) coincided with the period of venomotor contraction. It was also observed that the amount of splenic dilatation induced by stimulation of venomotor nerves was as great as that induced by contraction of the vein after direct application of the electrodes.

The Venomotor Path from the Cord.—In one of our early experiments with the plethysmograph it was noted that dilatation of the spleen occurred whenever the diaphragm was pulled upon. In other experiments a similar effect was produced by stimulation of the left phrenic nerve. Consequently the left phrenic nerve was carefully dissected in the lower part of its course. Near the point of junction of the main nerve with the diaphragm the nerve was found to send a perforating branch through the diaphragm. This branch accompanies the left inferior phrenic artery across the tendinous part of the diaphragm, and now runs longitudinally along the caudad surface of the latter. The inferior phrenic artery branches and gives off a twig to the left suprarenal gland, about 1 cm. proximal to which branching a nerve filament parts company with the artery to proceed to the dorsal aspect of the cœliac ganglion. This filament is so fine that the dissection had to be carried out repeatedly before one could be certain of its course. Examination under osmic acid seemed to show that the fibres of the filament in question are non-medullated. The fact that medullated fibres of the phrenic nerve, destined for the muscle of the diaphragm, also accompany the inferior phrenic artery for a considerable distance adds to the difficulty of identifying the sympathetic filament, which is covered by peritoneum and surrounded by the sheath of the artery on one side and by muscle fibres on the other. It may be most conveniently located on the lateral aspect of the artery about 2 to 3 cm. proximal to the suprarenal gland. It is interesting to note that Sir William Flower (1881) figured for the human body a special plexus of nerve fibres connecting the left phrenic nerve with the cœliac ganglion. We dissected the phrenic nerve in the neck on the left side, and found that a fine branch which seems to communicate with the sympathetic chain joins it at the level of the 6th cervical foramen.

The supposition that venomotor fibres to the splenic vein are carried by the phrenic nerve was tested and confirmed in three different ways.

Experiment.—Type I.

The spleen and its mesentery were freely exposed by the usual method of left-sided anterior thoracoplasty, with the incision carried along the infracostal border to the mid-line. By this means the splenic vein, having been stripped of its peritoneal covering, can be measured without interference with the surrounding tissues. The spleen, its surface protected from drying by means of a thin covering of absorbent cotton wrung out of warm saline, was placed in the warmed air plethysmograph, which is fixed in the required position by supports from the table. In sealing the plethysmograph with vaseined strips of cotton wool, care is taken to compress the vein as little as possible. A Marey tambour records on a drum gross changes in the size of the organ. A signal-marker connected with the induction coil records the period of faradic stimulation. A record of the carotid blood-pressure is meantime taken.

Shielded electrodes were affixed to the left phrenic nerve, and faradic stimulation of intensity just sufficient to cause contraction of the diaphragm was given at ten-minute intervals. The vein was measured with screw calipers before and after each stimulation, and its diameter recorded in corresponding positions on the drum. As a control, the effect of stimulation of the venomotor nerve peripherally was recorded also.

Stimulation of the phrenic nerve produced a gradual dilatation of the spleen, similar in amount and duration to that produced by stimulation of the nerve to the vein. At the same time the splenic vein contracted perceptibly. The results of four successive trials are given in tabular fashion. Stimulation of the venomotor filament to the primary division of the vein caused a contraction from 5·0 to 4·0 mm., with a plethysmographic rise of 7·0 mm., and gradual return to normal in two minutes. After this nerve filament had been removed from the vein the height and length of splenic dilatation were reduced on stimulation of the phrenic nerve.

Vein before stimulation .	6·0 mm.	6·0 mm.	6·5 mm.	6·0 mm.
Vein after stimulation .	4·5 ,,	4·5 ,,	5·0 ,,	5·0 ,,
Plethysmographic rise .	7·0 ,,	9·0 ,,	7·0 ,,	7·0 ,,
Duration of splenic dilatation	1½ min.	2 min.	2 min.	2 min.

Experiment.—Type II.

The spleen was exposed in the usual way, but the left thorax was opened throughout its whole length. To expose the left phrenic nerve to its origin, dissection was carried out in the neck. Smoked-paper impressions of the spleen surface were taken before and after stimulation of different portions of the phrenic,

simultaneously taken measurements of the splenic vein being recorded on the same paper.

First, the branch from the phrenic on the caudad aspect of the diaphragm was identified, and shielded electrodes were applied to it. Linear measurements of the diameter of the vein and of the spleen taken on four successive occasions were as follows:—

Vein before stimulation .	7·0 mm.	8·0 mm.	6·0 mm.	7·0 mm.
Vein after stimulation .	6·0 ,,	6·0 ,,	5·0 ,,	6·0 ,,
Spleen before stimulation .	65·0 ,,	60·0 ,,	59·0 ,,	55·0 ,,
Spleen after stimulation .	70·0 ,,	65·0 ,,	63·0 ,,	60·0 ,,

As a control the tissues accompanying the sub-diaphragmatic nerve were stimulated, and no effect was produced either on vein or on spleen. The nerve itself was again stimulated, with the result that the main vein contracted from 7·0 to 6·5 mm., and the primary division from 7·0 to 6·0 mm., with an increase in the diameter of the spleen from 52 to 56 mm.

Stimulation of the phrenic nerve in the neck at the level of the 7th cervical root produced on five successive occasions contraction of the vein, as recorded by caliper measurements, and dilatation of the spleen.

Stimulation of the 5th cervical root produced contraction of the vein and dilatation of the spleen, *e.g.* before stimulation the primary branch measured 6·5 mm., the main vein 7 mm., and the spleen 48 mm. in diameter; whereas after stimulation the primary branch measured 5·5 mm., the main vein 6·0 mm., and the spleen 50 mm.

Owing to the long duration of the experiment the spleen had gradually become contracted. When asphyxia of the animal was induced a marked further contraction occurred.

In order to discover whether the phrenic route is the sole path of venomotor fibres to the splenic vein, and whether these fibres are preganglionic or postganglionic, a series of degeneration experiments was carried out. These involved two stages: (1) aseptic section of the sub-diaphragmatic branch of the phrenic to the cœliac ganglion; (2) examination of the effects produced in the course of three to eight days. These two operations were done in each case under amytal anæsthesia and with the animal in the fasting condition.

Experiment.—Type III.

Stage A. February 26th.

The operation field was shaved and scrubbed, then disinfected with alcohol and iodine. Left anterior subcostal incision. The course of the venomotor fibres from the phrenic to the cœliac ganglion on the caudad surface of the diaphragm was recognised. The

peritoneum was incised between 3 and 4 cm. craniad to the suprarenal gland, and the nerve, with the accompanying artery, was separated from the diaphragm for a distance of 1 cm. It was doubly ligatured (including the vessel), and the intervening section removed. The cut edges of the peritoneum were approximated by a suture. Abdomen closed in four layers. Collodion dressing to wound. The dog was wrapped up and kept on a warm table until it had recovered from the anæsthetic.

The nerve-section removed was fixed in osmic acid, teased out, and examined.

Stage B. March 3rd (*i.e.* 5 days later).

The usual complete exposure by thoracoplasty was done. The spleen was smaller than it was at the previous operation. Stimulation of the sub-diaphragmatic extension of the phrenic below the point of section failed to produce any increase in size of the spleen. The main vein was then exposed in the usual way and was found to be abnormally dilated. A second stimulation of the aforesaid nerve failed to cause any contraction of the vein. The venomotor nerve was identified, and the shielded electrodes applied close to its insertion on the vein. One stimulation caused a very slight sluggish contraction. A second stimulation seemed to cause a slight dilatation of the vein. After this the vein seemed insensitive to venomotor stimulation, and the spleen showed no tendency to dilate. The experiment having been terminated by cutting off the artificial respiration, the section of the splenic vein, with its nerve attached, was fixed in osmic acid for examination.

The above observations were extended in other experiments. As early as the third day after section, stimulation of the phrenic in the thorax or below the operated area had little or no effect on the splenic vein. In every case the spleen was smaller than at the first operation, and during several hours of observation in each case failed to dilate to its original capacity. For instance, in one case eight days after nerve section the spleen had contracted from 10 in. by $3\frac{1}{2}$ in. to 7 in. by $2\frac{3}{4}$ in., and on any slight excitation there was an unusual tendency towards further contraction, which was unaffected by subsequent venomotor stimulation. The venomotor nerve was never found to contain medullated fibres.

Our provisional conclusion from these experiments is that the venomotor fibres to the splenic vein carried by the phrenic nerve are of the non-medullated variety, and pass through the cœliac ganglion without synapsing there. Moreover, physiological dilatation of the spleen is due to passage of venomotor impulses down these venomotor fibres. Altogether, apart from the general consideration that no other mechanism than the venous is apparent whereby the blood that enters under arterial pressure may be temporarily dammed up, the deduction follows

from these experimental facts: (1) On electrical stimulation of the venomotor fibres the resulting engorgement of the spleen corresponds in degree and duration to the contraction of the splenic vein; (2) when the venomotor fibres degenerate after section, the spleen remains in a state of partial contraction; (3) section of these fibres abolishes reflex contraction. MASUDA—see BARCROFT (1926)—has shown that central stimulation of the depressor trunk of the cut vagus induces splenic dilatation. We have found that this holds true only when the venomotor fibres are intact.

Visceromotor Reflexes involving the Splenic Nerve.—It has already been mentioned that MILLER and SIMPSON obtained visceromotor reflexes on stimulation of the splenic nerve. We devoted some time to an investigation of this matter. To begin with, certain novel and unrecorded phenomena in relation to the tone of the abdominal musculature in general deserve mention.

When the individual muscles that constitute the ventral abdominal wall (viz. *rectus abdominis*, *obliquus externus*, *obliquus internus*, and *transversalis*) are isolated and appropriately attached to heavy levers, each is found to be for the greater part of the time in slight tonic contraction. This contraction represents a postural tone. Clinicians palpating the abdomen are aware that the rigidity of the abdominal wall varies with the phase of respiration, hence the instruction to the patient to "take deep breaths." It was of interest to find that just at the moment of commencing inspiration the tone of each of the four muscles is abruptly inhibited, and remains inhibited just for a short period (fig. 4). Thus the physiological moment at which to palpate the abdomen deeply is at the commencement of inspiration.

A second peculiarity in the behaviour of these muscles is this. If any one of them is suddenly stretched it immediately responds with a contraction. In other words, it behaves like the *quadriceps extensor femoris* in the knee-jerk reflex. This helps to explain the rigidity that is apt to occur in the abdominal wall on forcible attempts to palpate deeply. Sudden stretching of any one muscle is sufficient to induce reflex contraction, not only of itself but also of all the other muscles on the same side. While stretching acts as a stimulus, it is not the only adequate stimulus, for squeezing any one muscle between the fingers suffices to elicit this conjoint reflex contraction of all the muscles of the same side.

A third peculiarity is this. Whenever one of the abdominal muscles on a particular side is induced reflexly to contract, the tone of the abdominal muscles on the opposite side is temporarily inhibited. This striking effect, verified over and over again, is just as definite and as invariable as the inhibition of tone observed by SHERRINGTON (1905), and by him named "reciprocal innervation" (fig. 5). Indeed, the relation between the muscles on opposite sides of the abdomen, so far as

this inhibitory reflex is concerned, is precisely the same as that existing between the flexor and extensor group at one of the joints of the limbs of the body.

The physiological purpose of this reciprocal arrangement is no doubt connected with the fact that the abdominal viscera have a certain (temporarily fixed) volume, which volume must at all times be easily accommodated within the space allotted to them. Should the animal curve its body, say, to the right, the abdominal muscles on that side,

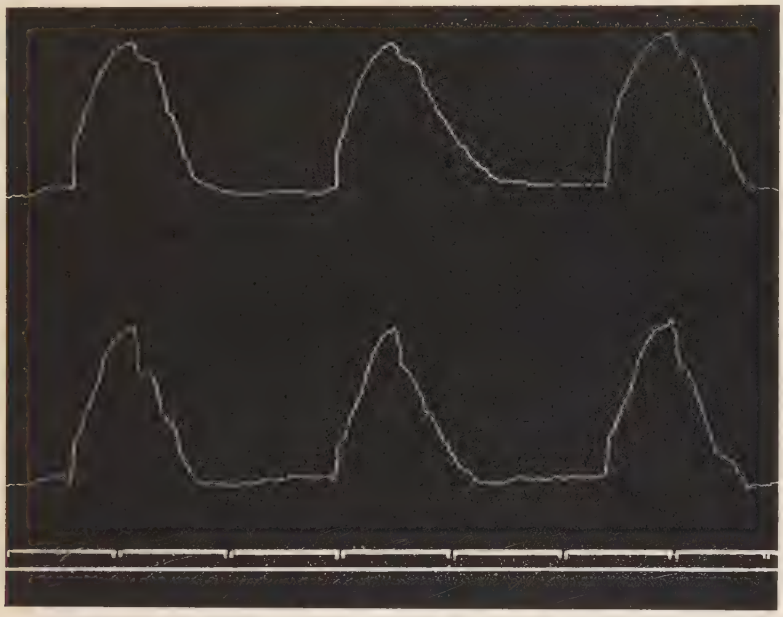

Fig. 4.—To illustrate temporary inhibition of tone of abdominal muscles during inspiration. The sternum was rigidly fixed in position. Time-record shows 2-second intervals. Relaxation of tone permits an upward movement of the attached lever. Anæsthetised dog.

The upper tracing is that of the craniad half of the left rectus muscle, the lower being that of the corresponding half of the right rectus. While tone tends to be maintained throughout at a uniform level, an abrupt inhibition of this tone, lasting for over a second, occurs at the commencement of each inspiration.

entering into rigid contraction, shorten and now pass in a straight line from point of origin to point of insertion. Thus the configuration of the somatic tube enclosing the viscera is changed. A simultaneous inhibition of the tone of the muscles on the opposite side will, under these conditions, allow of easy lateral shift of the viscera to the left, which adjustment may be accompanied by little or no (enduring) alteration of internal abdominal pressure. In this connection *cf.* G. H. Meyer (1861, 1873) and H. O. Feiss (1906). An analogous physiological provision, depending on the anatomical conformation of the

somatic segments and not upon reciprocal innervation of muscles, has been pointed out by Tait (1917) in the case of the abdominal region of the long-tailed decapod Crustacea.

Having stated these facts by way of preliminary, let us now consider the reflex responses of the abdominal muscles to electrical stimulation of the splenic nerve. It is a matter of common clinical experience

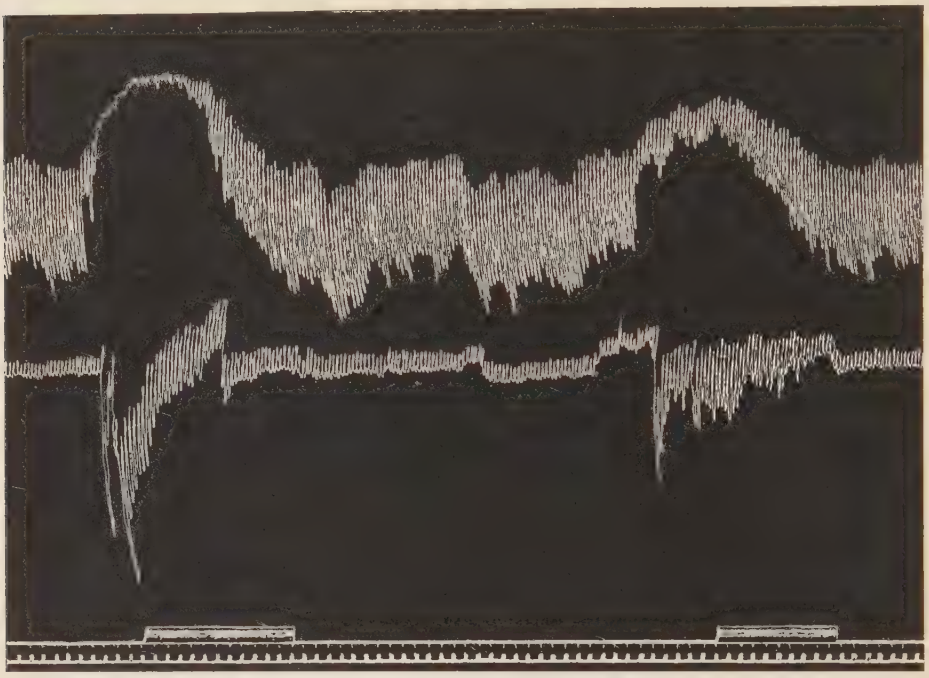

Fig. 5.—To illustrate reciprocal action between muscles on opposite sides of abdomen, and response of two selected abdominal muscles to stretching or squeezing of a third. Time-record shows 5-second intervals. Contraction is signalled in each case by a downward movement of the recording lever. The sternum was rigidly fixed in position. Anæsthetised dog.

The upper tracing is that of the craniad half of the left rectus. The lower tracing is that of the craniad portion of the right external oblique. The rhythmic movements of each muscle coincide with respirations, a relaxation of tone occurring with each inspiration.

Throughout the time of the first signal, steady tension was applied to a third muscle, viz. the right rectus. During the time of the second signal the same muscle was steadily squeezed between the fingers. The result in each case is an inhibition of the tone of the left rectus and an increase of tone of the right external oblique.

that different parts of the abdominal wall are maintained in a state of hypertonicity in disease of different viscera. In this case our object was to determine experimentally the regions of abdominal musculature affected by afferent impulses from the spleen. No matter whether one stimulates trunk C or either of the parallel branches of trunk A or of trunk B, the result is a marked increase of the tonic contraction of the abdominal muscles in the left upper quadrant, and a less marked increase

in tone of the muscles of the right upper quadrant of the abdomen. The musculature of the lower part of the abdomen, on the other hand, is not involved.

Experiment.

Under amytal anæsthesia the sheaths of the *recti* muscles on both sides were opened, and the muscles separated from the sheath ventrally and dorsally without damage to the nerves of supply. The muscles were in this case divided below the insertion of the 9th thoracic nerves. The external oblique, internal oblique, and transversalis muscles were similarly prepared on each side. The spleen was delivered, the splenic nerve was exposed and shielded electrodes applied to it. In order mechanically to fix the place of origin of the muscles, the sternum of the dog was rigidly clamped to a strong transverse metal bar fixed across the operating table. To the cut ends of the muscles, and in the direction of their fibres, strings were attached; by means of pulleys appropriately placed these strings were bent so as to pull on separate spring levers recording on the revolving drum. In any single case records of only two muscles were taken at a time, usually of corresponding muscles, right and left. Thus we recorded contractions of the two recti simultaneously, or of the two external obliques, and so on. Each time the splenic nerve was faradically stimulated the muscle of the upper left side went into a condition of increased tone. During the time of stimulation the respiratory oscillations of these muscles were inhibited, although the movement of the chest itself was unchanged. The tonic contraction of the muscle lasted somewhat longer than the period of stimulation, and was followed by a period of increased respiratory excursions of the muscles lasting for 1 to $1\frac{1}{2}$ minutes. A similar phenomenon, only less marked, was observed in the corresponding muscle of the right side. When the electrodes were shifted to any one of the individual trunks of the splenic nerve a similar effect occurred. We satisfied ourselves that the effect was not in any way due to physical spread of the stimulating currents. Finally, it was found that mechanical stimulation of the spleen itself, or traction on the splenic mesentery, produced a marked result on the left side, but a barely appreciable effect on the right side. As a control the liver was mechanically stimulated: in this case the muscles on the right side contracted markedly, while those on the left were barely affected.

When the levers were connected with the lower part of each abdominal muscle no contraction was recorded on stimulation of the spleen or of the splenic nerve. That these portions do respond to stimulation of the appropriate region was clear from the fact that mechanical stimulation of the small intestine, or traction on its mesentery, causes them to pass into tonic contraction.

The above recorded experiment, which was verified in different ways and at different times, is of clinical as well as of physiological interest.

It clearly establishes these two facts, viz. that the afferent nerves of the spleen are particularly connected with the abdominal musculature of the left upper quadrant, though also with that of the right upper quadrant; and that particular regions of the abdominal musculature

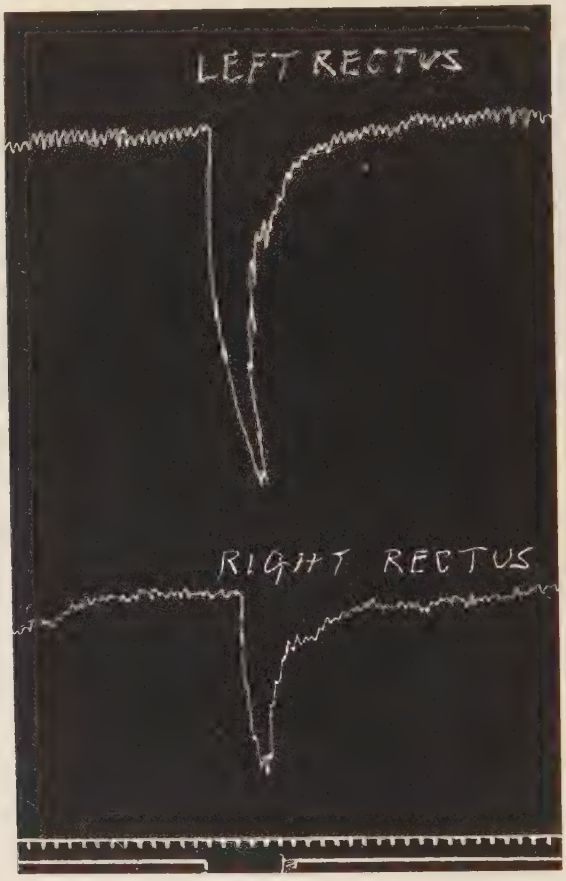

Fig. 6.—Splenic visceromotor reflex as shown by the craniad half of the left and of the right rectus muscle respectively. Spinal cat under artificial respiration. Sternum rigidly clamped in position. Contraction causes in each case a downward movement of the recording lever. Time-record shows 2-second intervals.

Slight respiratory oscillations are present. When the spleen is rubbed between the fingers the left rectus shows a pronounced contraction, the right rectus, after a longer latent period (the two levers were in accurate vertical alignment), a less pronounced contraction. During each of these contractions the respiratory oscillations are still present.

are responsive to stimulation of special visceral organs. It is quite possible that certain types of splenic disease will be found to be associated with hypertonicity, especially of the left upper quadrant of the abdomen.

Repetition of the above experiments with the more sensitive (unanæsthetised) spinal cat preparation (Sherrington, 1909) brought out

additional facts worthy of record, viz. (1) when single-induction break shocks are applied to the splenic nerve, the contrast between the strong response of the upper left muscles and the weak response of the upper right muscles is more striking; (2) the contrast between the responses of the external obliques is greater than that between the two recti;

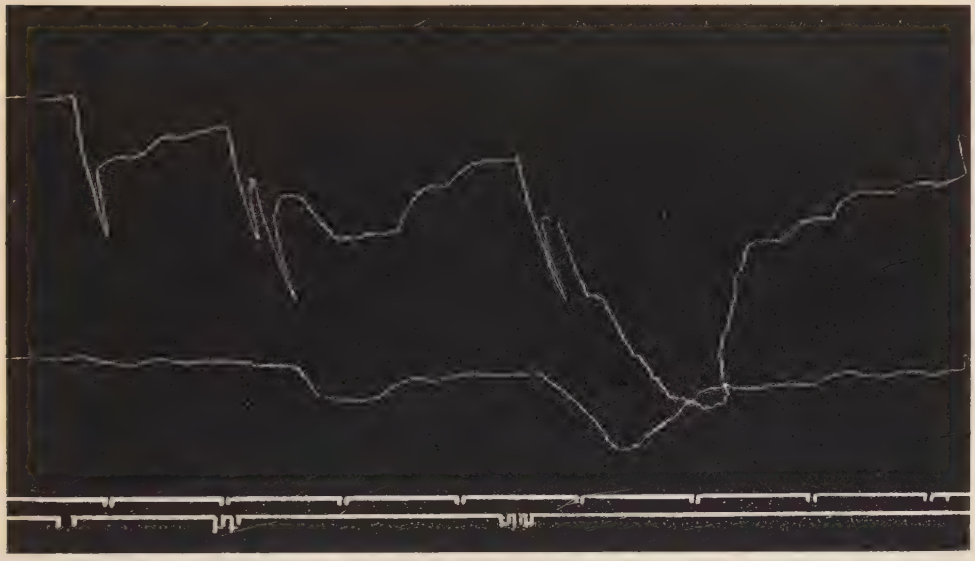

FIG. 7.—To illustrate the splenic visceromotor reflex as elicited by individual electrical excitations of the splenic nerve-trunk ; also to show the relative degree of response on the left as compared with the right side. Spinal cat under artificial respiration. Sternum rigidly clamped in position. Contraction causes in each case a downward movement of the recording lever. Time-record shows 2-second intervals. Each signal-mark below the time-record indicates the moment of application of one induction shock to the splenic nerve.

The upper tracing is that of the craniad half of the left rectus, the lower that of the same half of the right rectus. Respiratory oscillations are absent.

N.B.—(1) When the splenic nerve is excited once, the left rectus contracts, the right rectus meantime remaining as before ; (2) when the splenic nerve is excited twice in succession there is a more pronounced and longer contraction of the left rectus, while the right rectus also responds slightly ; (3) when three several excitations are applied to the nerve the effect upon each muscle is increased, the left again responding more markedly than the right ; (4) the latent period of response of the right rectus is longer than that of response of the left.

(3) by the use of weak single stimuli the effect may be confined to the muscles of the left upper quadrant, while summation of these stimuli may also give a response in the right; (4) the latent period of the response in the right muscles is always longer than the latent period of the response to splenic stimulation of the muscles on the left side. See figs. 6 and 7, and *cf.* SHERRINGTON (1906).

One point of incidental interest encountered in our experiments is the following. While making observations on the splenic visceromotor reflex we sometimes took simultaneous records also of splenic volume

and of blood-pressure. So soon as the splenic nerve is faradically excited the spleen begins to contract. After a momentary drop the systemic blood-pressure then rises, presumably owing to outpouring of the splenic blood. On cessation of the stimulation the spleen begins to dilate again, and the blood-pressure falls in unison with this dilatation. The rate of splenic dilatation does not proceed uniformly, however. As a rule it becomes slowed at a certain stage (fig. 8), which stage is

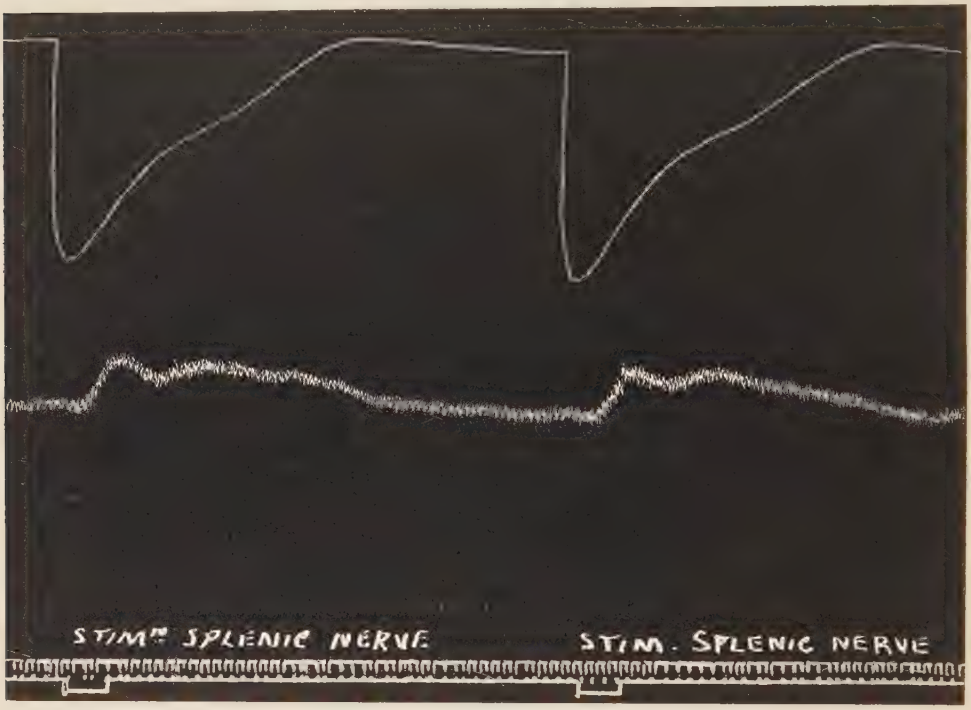

Fig. 8.—To show the simultaneous effect on the splenic volume and on the systemic blood-pressure of stimulating the intact splenic nerve. Amytalised dog. In the upper record, which shows change of splenic volume, a downward movement means contraction of the spleen. Time-record shows 2-second intervals. For further description see text.

accompanied by a second rise of blood-pressure. In cases where this pronounced lag in the rate of splenic dilatation is absent, as when stimulation of the splenic nerve is prolonged, there is no corresponding second rise of blood-pressure. We have not further analysed this interesting phenomenon, which naturally suggests comparison with the effect on blood-pressure recorded by Elliott (1912) and others as a result of stimulating the splanchnic nerve. For a discussion of this latter effect, with full references to the literature, see Sharpey Schafer (1924).

While Schäfer and Moore (1896) were able to demonstrate a very extensive origin of efferent fibres from the cord to the spleen, no one

has as yet attempted to determine by which spinal nerve roots the afferent fibres enter. It was conceived that this problem could be solved by using the visceromotor reflexes in the following way, two animals being required in each case.

Experiment. Animal 1.

Under ether anæsthesia tracheotomy was performed on a cat. The carotids and the vagi were tied; the vertebral arteries were compressed by a ligature; the cord was transected between the foramen magnum and the atlas; and artificial respiration was instituted. The right and left external obliques were then prepared in the usual way. The cat was now placed in the prone position, and a narrow laminectomy was performed, reaching from above the 4th thoracic to below the 4th lumbar vertebræ. In order to make sure that the whole reflex arc was intact, the animal was laid in the supine position, the splenic nerve was stimulated, and a contraction of the two external oblique muscles was recorded. With the animal on its right side the dura mater was now longitudinally incised. When the edges of the dura were lifted up, one obtained a view of the series of dorsal nerve roots attached to the cord. In successive order from the 4th lumbar upwards these roots were divided on the left side. After section of each root, a test was made for the presence of the visceromotor reflex of the left external oblique muscle, recording the effect upon the drum. Only when the dorsal root of the 5th thoracic segment was divided was the reflex abolished. To mechanical stimulation of successive left ventral nerve roots from the 5th thoracic to the 3rd lumbar inclusive, the muscle still responded on each occasion with contraction.

The animal having been laid on its left side, the right external oblique was now attached to the recording lever. No stimulation of the splenic nerve was able to cause a contraction of this muscle. That the efferent nerve roots to the muscle were intact was proved by mechanical stimulation of the successive nerve roots.

It follows from the above experiment, which was repeated two or three times with the same result, that the afferent fibres of the visceromotor reflex arc enter at a level below the 4th thoracic segment and, whether the effector organ be on the left or on the right side, they enter only by way of left dorsal nerve roots.

Experiment. Animal 2.

The same essential operation was performed as in the case of animal 1, with this difference, that the left dorsal nerve roots were successively divided *from before backwards*, commencing with the 4th thoracic. The visceromotor reflex involving the left external

oblique remained present until the 8th thoracic was cut. Mechanical stimulation of anterior nerve roots showed that the efferent side of the arc was still intact. The animal having been turned on its left side, and the right external oblique having been connected with the lever, no response of the muscle followed stimulation of the splenic nerve. At the same time, contraction of the muscle followed stimulation of the anterior nerve roots.

Taking this experiment in conjunction with the preceding, we conclude that the afferent fibres of the splenic visceromotor reflex enter the cord by the 5th, 6th, 7th, and 8th dorsal thoracic roots, and that they pass in only by way of left dorsal nerve roots.

Summary of Conclusions.

1. The splenic nerve of the dog contains at least two sets of efferent fibres, distinct in their anatomical arrangement and in the form of contraction they induce, the one set being probably arteriomotor, the other trabeculomotor.

2. Afferent fibres are present in all trunks of the splenic nerve.

3. Certain discrete segments of the splenic vein and of its main branches are endowed with special contractility. The segments in question form the terminal stations of particular nerve-twigs from a main venomotor trunk, electrical stimulation of which thus causes local bands of contraction in the specially innervated regions.

4. The splenic venomotor fibres join the left phrenic nerve in the mid-cervical region. Coursing with it as non-medullated fibres, they eventually perforate the diaphragm, where for a time they accompany the inferior phrenic artery. Deviating towards the cœliac ganglion, they next join company with the splenic vein, and are eventually distributed to localised parts of the vein.

5. Physiological dilatation of the spleen is due to passage of nervous impulses down these venomotor fibres. This conclusion follows from the fact that on electrical stimulation of these fibres the resulting engorgement of the spleen corresponds in degree and in duration to the contraction of the splenic vein; from the further fact that when the venomotor fibres degenerate after section, the spleen remains in a state of partial contraction; and from the fact that section of these fibres abolishes reflex dilatation. The venomotor fibres thus form the efferent pathway for physiological dilatation.

6. The muscles of the ventral abdominal wall are maintained in a condition of steady postural tone, which, however, is momentarily inhibited at the commencement of each inspiration.

7. When any given abdominal muscle is suddenly stretched or squeezed, both it and the other abdominal muscles on the same side

exhibit a strong reflex contraction. At the same time the tone of the abdominal muscles of the opposite side is by reciprocal action temporarily inhibited.

8. Increased tonic contraction, unaccompanied by inspiratory relaxation, occurs in particular regions of the ventral abdominal musculature as a result of effective stimulation of particular viscera through their afferent nerve fibres. The portion of this musculature innervated from the 9th thoracic segment upwards on the left side is particularly responsive to stimulation of the afferent nerve fibres of the splenic nerve. Under more powerful stimulation of the splenic afferents, the region involved in reflex contraction may extend from this special splenic area to a correspondingly delimited area on the opposite side. The above observations may well have a corresponding clinical application.

9. The afferent fibres for the splenic visceromotor reflex enter the spinal cord by the 5th, 6th, 7th, and 8th dorsal roots and are confined to the left side.

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